

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460



OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: September 20, 2019

SUBJECT: **Ethoxyquin:** DERs for relevant studies from the open literature and unpublished studies funded by NTP that are referenced in the registration review human health risk assessment

PC Code: 055501

Decision No.: 549718

Petition No.: N/A

Risk Assessment Type: N/A

TXR No.: 0057944

MRID No.: See Table 1

DP Barcode: D454047

Registration No.: N/A

Regulatory Action: N/A

Case No.: N/A

CAS No.: 91-53-2

40 CFR: §180.178

FROM: Austin Wray, Ph.D., Toxicologist
Risk Assessment Branch IV,
Health Effects Division (7509P)

Handwritten signature of Austin Wray in black ink.

THROUGH: Monique Perron, Ph.D., Acting Branch Chief
Risk Assessment Branch IV
Health Effects Division (7509P)

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TO: Brian Van Deusen, Risk Assessor
Risk Assessment Branch IV,
Health Effects Division (7509P)

I. ACTION REQUESTED:

The ethoxyquin human health risk assessor requested the lead toxicologist compose abbreviated data evaluation records (DER) for relevant studies from the open literature and unpublished studies funded by the National Toxicology Program (NTP) to support the ethoxyquin registration review.

II. BACKGROUND:

In addition to guideline and non-guideline studies submitted by the registrant, the hazard characterization for ethoxyquin relies, in part, on published literature and unpublished studies funded by NTP. Brief summaries for several of the published studies were presented in the Registration Eligibility Decision (RED; 2004); however, the review of these studies was not captured in a DER. Abbreviated DERs were created to summarize the literature studies and unpublished NTP studies (MRIDs 05000475, 05012507, 50920701-12, and 50938001-02) that were determined to be of sufficient quality based on the 2012 OPP open literature guidance and reported unique information that was incorporated into the hazard characterization.

III. RESULTS/DISCUSSION:

The title and MRID number of studies that required abbreviated DERs for the ethoxyquin registration review are listed in Table 1 below. Refer to the attached DERs for more information on the results presented in the relevant publications and unpublished studies funded by NTP.

Table 1. Citation and MRIDs of studies that are summarized in the attached abbreviated DERs


Citation	MRID
Wilson RH, JO Thomas, CR Thompson, HF Launer, and GO Kohler. 1959. Absorption, metabolism, and excretion of the antioxidant, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline. <i>Agricultural and Food Chemistry</i> . 7(3) 206-209.	05000475
Takahashi O and K Hiraga. 1978. The relationship between hemorrhage induced by butylated hydroxytoluene and its antioxidant properties or structural characteristics. <i>Toxicol Appl Pharmacol</i> . 46(3): 811-814.	05012507
Burka LT, JM Sanders, and HB Matthews. 1996. Comparative metabolism and disposition of ethoxyquin in rat and mouse. II. Metabolism. <i>Xenobiotica</i> . 26(6) 597-611.	50920701
EHRT. 1993. Range-finding studies: Developmental toxicity ethoxyquin when administered via gavage in CD Sprague-Dawley rats. Environmental Health Research and Testing, INC. (EHRT) Lexington, KY. Study Number: NTP-92-RF/DT-040. April 1993. Unpublished.	50920702
EHRT. 1993. Range-finding studies: Developmental toxicity of ethoxyquin when administered via gavage in New Zealand white rabbits. Environmental Health Research and Testing, INC. (EHRT) Lexington, KY. Study Number: NTP-92-RF/DT-045. April 1993. Unpublished.	50920703
Hard GC and GE Neal. 1992. Sequential study of the chronic nephrotoxicity induced by dietary administration of ethoxyquin in Fischer 344 rats. <i>Fundamental and Applied Toxicology</i> . 18: 278-287.	50920704
Khera KS, C Whalen, G Trivett, and G Angers. 1979. Teratologic assessment of maleic hydrazide and diaminozide, and formulations of ethoxyquin, thiabendazole and naled in rats. <i>Journal of Environmental Science & Health Part B</i> . 14(6): 563-577.	50920705
Manson MM, JA Green, BJ Wright, and P Carthew. 1992. Degree of ethoxyquin-induced nephrotoxicity in rat is dependent on age and sex. <i>Arch Toxicol</i> . 66: 51-56.	50920706
Manson MM, JA Gree, and HE Driver. 1987. Ethoxyquin alone induces preneoplastic changes in rat kidney whilst preventing induction of such lesions in liver by aflatoxin B ₁ . <i>Carcinogenesis</i> . 8(5): 723-728.	50920707

Citation	MRID
Neal GE, DJ Judah, GG Hard, and N Ito. 2003. Differences in ethoxyquin nephrotoxicity between male and female F344 rats. Food and Chemical Toxicology. 41: 193-200.	50920708
Sanders JM, Burka LT, and Matthews HB. 1996. Comparative metabolism and disposition of ethoxyquin in rat and mouse. I. Disposition. Xenobiotica. 26(6): 583-595.	50920709
Skaare JU and I Nafstad. 1979. The distribution of ¹⁴ C-ethoxyquin in rat. Act Pharmacol et Toxicol. 44: 303-307.	50920710
Skaare JU and E Solheim. 1979. Studies on the metabolism of the antioxidant ethoxyquin, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline in the rat. Xenobiotica. 9(11): 649-657.	50920711
Skaare JU. 1979. Studies on the biliary excretion and metabolites of the antioxidant ethoxyquin, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline in the rat. Xenobiotica. 9(11): 659-668.	50920712
Ørnsrud R, A Arukwe, V Bohne, N Pavlikova, and A-K Lundebye. 2011. Investigations on the metabolism and potentially adverse effects of ethoxyquin dimer, a major metabolite of the synthetic antioxidant ethoxyquin in salmon muscle. Journal of Food Protection. 74(9): 1574-1580.	50938001
Bernhard A, JD Rasinger, H Wisløff, Øyvør Kolbjørnsen, LS Myrmel, MHG Berntssen, A-K Lundebye, R Ørnsrud, and L Madsen. 2018. Subchronic dietary exposure to ethoxyquin dimer induces microvesicular steatosis in male BALB/c mice. Food and Chemical Toxicology. 118: 608-625.	50938002

IV. CONCLUSIONS:

In total, abbreviated DERs were composed for 14 studies from the open literature and two unpublished studies sponsored by NTP. All of these studies met the acceptability criteria based on the 2012 OPP literature guidance and contributed unique information to the ethoxyquin human health risk assessment that was not captured in the toxicity database. The findings presented in these studies, however, did not have a quantitative impact on the ethoxyquin registration review risk assessment.

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (% a.i. not reported)
[¹⁴C]-ethoxyquin (95% purity)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Wilson RH, JO Thomas, CR Thompson, HF Launer, and GO Kohler. 1959.
Absorption, metabolism, and excretion of the antioxidant, 6-ethoxy-1,2-dihydro-
2,2,4-trimethylquinoline. Agricultural and Food Chemistry. 7(3) 206-209. MRID
05000475.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a metabolism study (MRID 05000475), unlabeled ethoxyquin (% a.i. not reported but assumed to be similar to the labeled ethoxyquin; Monsanto Chemical Co.) was administered in the diet to 3 albino rats/sex at a dietary concentration of 0.005% (estimated at 2.5 mg/kg/day assuming 1 ppm is equivalent to 0.05 mg/kg/day in rats in diet) for several weeks followed by either a single oral dose 1.5 mg (Table 3 indicates dose ranged from 6.7-9.2 mg/kg bw) of ¹⁴C-ethoxyquin (95% purity; Monsanto Chemical Co.) and sacrificed 1, 2, 7 days for females and 7, 14, 28 days for males, Respired air, urine and feces were collected for up to 4 and 7 days after the single oral dose in males and females, respectively, and tissues were analyzed for radioactive content after sacrifice. In another experiment, 2 albino male rats and 2 albino pregnant female rats were administered unlabeled ethoxyquin at a dietary concentration of 0.005% for several weeks then switched to a diet of 0.005% ¹⁴C-ethoxyquin for 10 days (study authors report daily intakes of 2.3-3.7 mg/kg bw). At the end of the exposure period, respired air samples were taken from the male rats and then they were sacrificed for tissue analysis. The pregnant rats delivered 9 days after switching to the labeled diet. Immediately after delivery and one day after delivery, 1 pup/litter was sacrificed, and gastrointestinal tract was removed to assess placental transfer. Both dams were also sacrificed the day after delivery for tissue analysis.

Elimination was rapid, with 52-84% of the administered single dose (AD) of labeled ethoxyquin excreted in the first 24 hours. Nearly all of the AD was eliminated four and seven days after the single dose exposure in males (80-92% of the AD) and females (95% of the AD), respectively. Urinary excretion was the primary route of elimination and accounted for 30-63% of the AD whereas fecal elimination accounted for 16-40% of the AD. Ethoxyquin was also measured in


expired air of females but accounted for <1% of the AD. Ethoxyquin-derived radioactivity was observed in tissues with the highest concentration observed in liver and kidneys. Ethoxyquin radioactivity was also observed in fat, spleen, heart, muscle, brain, and blood. In total, tissue radioactivity accounted for 0.2-1.9% of the AD. Tissue concentration declined over time after the single dose exposure; however, elimination from fat was slower than the other tissues examined.

Tissue distribution after 10 days of dietary exposure was similar to the single dose exposure. The highest ethoxyquin concentration was observed in the liver and kidneys, with lower but still quantifiable levels observed in heart, fat, muscle, brain, spleen, and blood. Low concentrations (0.12-0.19 ppm) of ethoxyquin were also quantified in milk collected from the pregnant dams. Pups sacrificed immediately after birth (no access to milk) and one day after birth exhibited similar ethoxyquin concentrations. The authors concluded that this finding along with the low ethoxyquin concentration in the milk indicates the tissue concentration in the pups was due to placental transfer and not exposure from milk. It should be noted that this conclusion and the milk concentration results are based on a small sample size (n=2). Consequently, the reviewer has lower confidence in these findings.

This non-guideline metabolism study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a metabolism study [OCSPP 870.7485, OECD 417].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity study; dietary- rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (101% a.i.)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Takahashi O and K Hiraga. 1978. The relationship between hemorrhage induced by butylated hydroxytoluene and its antioxidant properties or structural characteristics. Toxicol Appl Pharmacol. 46(3): 811-814. MRID 05012507.

SPONSOR: None reported.


EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 05012507), ethoxyquin (101% a.i.; Tokyo Kasei Kogyo Co.) was administered to 10 male Sprague-Dawley rats/dose in the diet at dose levels of 0 or 3.15 mmol/kg/day (equivalent to 0 or 652 mg/kg/day) for 3 weeks. Animals were monitored over the three week period and signs of intoxication, mortalities, and body weights were recorded. At the end of the exposure period, all rats were examined for signs of hemorrhage and the prothrombin index was calculated. Additional treatment groups were included in the experiment to assess toxicity from dietary exposure to Ionox330, butylated hydroxyanisole (BHA), 2,5-di-tert-butylhydroquinone (DBH), γ,γ -diphenyl-p-phenylenediamine (DPPD), 2,6-di-tert-butylphenol (DBP), butylated hydroxytoluene (BHT), BHT-alcohol, BHT-acid, BHT-aldehyde, 2,4,6-tri-tert-butylphenol (TBP), or 2,4,6-trimethylphenol (TMP). These additional treatment groups do not provide information that is relevant to the ethoxyquin risk assessment, thus the results are not discussed in this data evaluation record.

No clinical signs of toxicity in the treatment group were reported nor any treatment related effects on body weight or mortality rate. Ethoxyquin treated rats exhibited an overall significant reduction in prothrombin index (\downarrow 40% from control levels) and several rats from the treatment group (3/10) exhibited haemorrhaging in epididymis or testes.

This non-guideline subchronic study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSP 870.3100; OECD 408].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (90% a.i.)
3-[¹⁴C]-ethoxyquin (>96% radiolabeled purity)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Burka LT, JM Sanders, and HB Matthews. 1996. Comparative metabolism and disposition of ethoxyquin in rat and mouse. II. Metabolism. *Xenobiotica*. 26(6) 597-611. MRID 50920701.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a metabolism study (MRID 50920701), 3-[¹⁴C]-ethoxyquin (>96% radiolabeled purity; Amersham Corporation) diluted with unlabeled ethoxyquin (90% a.i.; Sigma Chemical Co.) in a vehicle (1:1:8 ratio of ethanol/emulphor EL-620/water) was administered to 2 month old male F344 rats and B6C3F1 mice (three rodents/treatment) as a single dose via gavage at 2.5 (rats only), 25, or 250 mg/kg or intravenous (i.v.) injection at 25 mg/kg. The 250 mg/kg dosing solution did not include the vehicle. A separate group of male rats (4 rats/treatment) were also orally exposed to six consecutive daily gavage doses of either 25 or 250 mg/kg. With a few exceptions, the authors of this study did not state the sampling intervals for the excreta and tissue metabolite analysis. The authors indicated the same rats from the Sanders *et al.* 1996 study (MRID 50920709) were used for this study; therefore, the reviewer assumed the excreta and tissue samples collected for that study were also available for metabolite analysis in instances where the sampling interval was not stated explicitly. Urine and feces were collected for 4 to 72 hours after dosing in the single dose experiment, and at 24 hours after the last dose in the repeat dose experiment. A subset of animals from the gavage (24 and 72 hours) and i.v. (0.25 to 24 hours) treatment groups were euthanized at various timepoints after exposure for tissues analysis. Bile was analyzed in a separate experiment. Three to five month-old F344 bile cannulated rats (3 rats/treatment) were exposed to a single i.v. dose of 25 mg/kg and bile was collected for 6 hours after administration for analysis.

Four urinary metabolites were identified in rats. The sulphate conjugates of 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline (metabolite G) and 1,2-dihydro-3,6-dihydroxy-2,2,4-trimethylquinoline (metabolite E) accounted for >50% of the radioactivity in the urine. The other two identified metabolites were glucuronide conjugates of 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline (metabolite F) and 1,2-dihydro-6-ethoxy-8-hydroxy-2,2,4-trimethylquinoline

(metabolite H), and were minor contributors to the radioactivity compared to the sulphate conjugates. The radiochromatogram results also indicated that four other minor metabolites were present in the urine samples; however, these metabolites but could not be isolated at a purity and quantity to allow for identification. Route of administration (e.g. oral v. intravenous) did not change the urine metabolic profile in rats. Three major biliary metabolites were identified in rats, all of which were glutathione conjugates. These metabolites include two glutathione conjugates of the 3,4-epoxide (metabolite 1 and 2) and either the 8- or 7-(S-glutathionyl)-2,2,4-trimethylquinol-6-one isomer (metabolite 3). No minor metabolites were identified in the bile from rats. The author presented proposed metabolic pathways for the identified urine and bile metabolites in Figure 7 and 8 in the publication.

The study authors indicate that extraction of ethoxyquin radioactivity from fecal samples was low ($30 \pm 7\%$) which limited metabolite identification. A compound that co-eluted with one of glutathione conjugates of the 3,4-epoxide (metabolite 2) identified in the bile accounted for 20-35% of the extracted radioactivity in the feces. No parent was observed in the extracted fecal samples from rats exposed via gavage to 25 mg/kg; however, a compound that co-eluted with parent accounted for 5% of the radioactivity in extracted fecal samples from rats exposed intravenously. Fecal samples from rats exposed to 250 mg/kg contained a compound with a similar retention time as the sulphate conjugate of 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline (metabolite G) noted in the urine. Kidney tissue also contained a compound with similar retention time to the sulphate conjugate of 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline (metabolite G) along with several minor metabolites that were not identified. Two major metabolites were observed in the liver. The study authors indicated that one of the metabolites was the sulphate conjugate of 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline (metabolite G) and the other was either the sulphate conjugate of 1,2-dihydro-3,6-dihydroxy-2,2,4-trimethylquinoline (metabolite F) or a glutathione conjugate of the 3,4-epoxide (metabolite 1) identified in the bile.


Dose and the dosing regimen both affected ethoxyquin metabolism in rats. An increase in the dose from 25 mg/kg to 250 mg/kg resulted in an increase in the proportion of the sulphate conjugate of 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline (metabolite G) and the glucuronide conjugate of 1,2-dihydro-6-ethoxy-8-hydroxy-2,2,4-trimethylquinoline (metabolite H) in the urine and a decrease in the proportion of the other metabolites. Repeated oral exposure to 250 mg/kg also resulted in a shift toward more glucuronide conjugates and less sulphate conjugates. In contrast, the metabolic profile in the urine of rats exposed to 25 mg/kg did not change significantly with repeated exposure.

Ethoxyquin metabolism differed significantly between the two rodent species based on the metabolite profile in the urine after a single i.v. dose of 25 mg/kg. The major metabolites in mouse urine were the sulphate and glucuronide conjugates of 1,2-dihydro-6-hydroxy-2,2,4-trimethylquinoline (metabolites G and F). Minor metabolites include the glucuronide conjugate of 1,2-dihydro-6-ethoxy-8-hydroxy-2,2,4-trimethylquinoline (metabolite H) as well as two of the unidentified minor metabolites in the rat urine. Both glucuronide conjugates were present in higher quantities in mouse urine compared rat urine indicating glucuronidation was a more prevalent metabolic pathway in mice. Parent was not present in the urine of either rats or mice following i.v. exposure.

This non-guideline metabolism study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a metabolism study [OCSPP 870.7485, OECD 417].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Prenatal developmental toxicity study - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (94% a.i.)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: EHRT. 1993. Range-finding studies: Developmental toxicity ethoxyquin when administered via gavage in CD Sprague-Dawley rats. Environmental Health Research and Testing, INC. (EHRT) Lexington, KY. Study Number: NTP-92-RF/DT-040. April 1993. Unpublished. MRID 50920702.

SPONSOR: National Toxicology Program (NTP)

EXECUTIVE SUMMARY: In a prenatal developmental toxicity study (MRID 50920702), ethoxyquin (94% a.i.; Raschig Corp) was administered to 8-11 CD (SD)BR rat dams/dose via oral gavage (in corn oil vehicle) at dose levels of 0, 100, 400, 600, 800, or 1000 mg/kg/day from gestation day (GD) 6 through 15, inclusive, of gestation. Dams were monitored for clinical signs of toxicity or mortality daily, and body weight was recorded throughout the exposure period and on the day of the cesarean section (GD 20). Dams were sacrificed on GD 20 and the following cesarean parameters were assessed: number of implantation sites, number of resorptions, number of dead fetuses, number of live fetuses, gravid uterine weight, and liver litter weight. Study authors did not assess fetal malformations or variations.

Clinical signs of toxicity including rough hair coat, sedate behavior, and blue urine and feces were observed in all dams exposed to 400 mg/kg/day. Clinical signs of toxicity were also noted in all animals from the 600, 800, and 1000 mg/kg/day treatment groups. They included some of the same signs observed at 400 mg/kg/day as well as more severe effects (e.g. lethargy or ataxia, salivation, hunched posture, chromodacryorrhea, lying flat or on side, moribund). The increase in severity of clinical signs corresponded with a dose dependent increase in the number of maternal mortalities at dose levels ≥ 600 mg/kg/day. Four mortalities were observed in the 600 mg/kg/day group and occurred on GD 9 and 10, with total group loss on GD 9 in the 800 and 1000 mg/kg/day treatment groups. Maternal body weight was significantly decreased from controls during and after exposure in the 400 and 600 mg/kg/day treatment groups ($\downarrow 9-16\%$), and prior to the total group mortality in the 800 and 1000 mg/kg/day treatment groups ($\downarrow 10-12\%$). No maternal effects were observed in dams from the 100 mg/kg/day treatment group.

A non-significant 11% decrease in mean fetal weight was observed in the 600 mg/kg/day treatment group. Although this finding is ostensibly a developmental effect, the declining health of the dams at this dose may have contributed to the lower fetal weight. There were no treatment related effects on any of the other caesarean parameters assessed in the 100, 200, 400, and 600 mg/kg/day groups. None of the dams from the 800 and 1000 mg/kg/day survived to the caesarean.

The maternal LOAEL is 400 mg/kg/day based on clinical signs of toxicity and decreased body weight. The maternal NOAEL is 100 mg/kg/day.

The developmental LOAEL is 600 mg/kg/day based on decreased fetal body weight. The developmental NOAEL is 400 mg/kg/day.

This non-guideline prenatal developmental toxicity study is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a prenatal developmental toxicity study in rats [OCSPP 870.3700a; OECD 414].

COMPLIANCE: A signed and dated Quality Assurance was provided and indicated the study was conducted in compliance with GLP's.

Table 1. Maternal mortality^{ab}						
	Dose in mg/kg bw/day					
	Control	100	400	600	800	1000
# dead/total # pregnant dams	0/11	0/11	0/8	4/10	10/10	8/8 ^c

^a Data obtained from page 12 in the study report^b Data presented for pregnant females only.^c 2 nonpregnant dams also died during the exposure period.

Table 2. Mean (±SE) maternal body weight and body weight gain (g)^{ab}						
Interval	Dose in mg/kg bw/day (# of Dams)					
	Control (11)	100 (11)	400 (8)	600 (6-10)	800 (8-10)^d	1000 (3-8)^d
Body Weight (g)						
Day 5	263.2±3.1	263.9±2.4	262.2±2.9	262.4±2.6	262.2±2.6	261.6±4.2
Day 6	271.5±2.6	268.6±2.5	266.7±2.6	269.8±2.7	269.7±4.1	269.8±4.6
Day 8	281.7±2.9	274.4±2.1	256.2±5.3* (↓9%)	253.5±2.5* (↓10%)	253.4±4.2* (↓10%)	248.7±1.6* (↓12%)
Day 10	295.9±3.6	289.9±2.5	261.2±8.5* (↓12%)	249.0±7.5* (↓16%)	--	--
Day 12	311.8±4.1	306.9±2.5	271.3±9.6* (↓13%)	260.6±9.5* (↓16%)	--	--
Day 14	327.8±4.1	321.6±2.8	282.6±8.8* (↓14%)	276.3±8.1* (↓16%)	--	--
Day 16	349.0±4.4	339.6±3.5	301.1±9.2* (↓14%)	292.5±7.5* (↓16%)	--	--
Day 20	416.3±5.6	408.9±7.1	363.6±11.4* (↓13%)	366.4±7.0* (↓12%)	--	--
Body Weight Gain (g)						
Treatment: Days 6-16	77.5±3.4	71.0±1.6	34.4±7.6* (↓56%)	25.1±9.5* (↓68%)	--	--
Gestation: Days 5-20	69.7±4.3	66.7±2.8	30.3±4.9* (↓57%)	27.8±6.3* (↓60%)	--	--
Gestation (corrected): Day 5-20^c	153.1±4.9	145.0±6.1	101.4±9.2* (↓34%)	107.4±9.0* (↓30%)	--	--

^a Data obtained from Tables 2 and 3 on pages 23 and 25 in the study report; values in parentheses are % change from controls calculated by the Reviewer.^b Data presented for pregnant females only.^c Gestation maternal body weight gain corrected for gravid uterine weight.^d All dams in the 800 and 1000 mg/kg/day treatment groups died prior to Day 10.

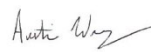
* Statistically different (p < 0.05) from the control.

Table 3. Cesarean section observations (mean±SE) ^a						
Observation	Dose (mg/kg bw/day)					
	0	100	400	600	800 ^b	1000 ^b
# Animals assigned (mated)	11	11	10	10	10	10
# Animals pregnant	11	11	8	10	10	8
Pregnancy rate (%)	100	100	80	100	100	80
# Nonpregnant	0	0	2	0	0	2
Maternal wastage						
No. died	0	0	0	4	10	10
No. died pregnant	0	0	0	4	10	8
No. died nonpregnant	0	0	0	0	0	2
No. aborted	0	0	0	0	0	0
No. Premature delivery	0	0	0	0	0	0
No. implantations/dam	14.5±0.3	13.6±0.6	13.1±1.4	14.5±0.4	--	--
No. non-live implantations/dam	0.6±0.5	0.8±0.3	0.9±.2	0.5±0.2	--	--
Total No. litters	11	11	8	6	--	--
No. live fetuses/dam	13.9±0.4	12.8±0.7	12.3±1.3	14.0±0.5	--	--
No. dead fetuses/dam	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.2		
Resorptions/dam	0.6±0.5	0.8±0.3	0.9±0.2	0.3±0.2	--	--
Litters with total resorptions	0	0	0	0	--	--
Mean fetal weight (g)	4.06±0.09	4.14±0.06	3.83±0.09	3.60±0.21 (↓11%)	--	--
Gravid uterine weight (g)	83.4±2.3	78.3±4.7	71.2±7.0	79.6±4.4	--	--

^a Data obtained from Table 4, page 27 in the study report; values in parentheses are % change from controls calculated by the Reviewer.

^b No caesarean section data due to total group loss

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Prenatal developmental toxicity study - rabbit; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (93.7% a.i.)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: EHRT. 1993. Range-finding studies: Developmental toxicity of ethoxyquin when administered via gavage in New Zealand white rabbits. Environmental Health Research and Testing, INC. (EHRT) Lexington, KY. Study Number: NTP-92-RF/DT-045. April 1993. Unpublished. MRID 50920703.

SPONSOR: National Toxicology Program (NTP)

EXECUTIVE SUMMARY: In a prenatal developmental toxicity study (MRID 50920703), ethoxyquin (93.7% a.i.; Raschig Corp) was administered to 9-10 New Zealand White rabbit does/dose via oral gavage (in corn oil vehicle) at dose levels of 0, 50, 200, 400, 600, or 800 mg/kg/day from gestation Day (GD) 6 through Day 19, inclusive. Does were monitored for clinical signs of toxicity or mortality daily, and body weight was recorded throughout the exposure period and on the day of the cesarean section (GD 30). Does were sacrificed on GD 30 and the following cesarean parameters were assessed: number of implantation sites, number of resorptions, number of dead fetuses, number of live fetuses, gravid uterine weight, and liver litter weight. Study authors did not assess fetal malformations or variations.

Clinical signs of toxicity including thin and moribund appearance were observed in several does from the 200 mg/kg/day. The same clinical signs were observed at dose levels ≥ 400 mg/kg/day along with low incidence of other signs including sedate behavior, lethargy or ataxia, soft stool, and diarrhea. In addition, three animals from the 200 mg/kg/day treatment group aborted prior to caesarean section (GD25 for one doe, exact day of abortion not reported for other two does though blood on cage pad was noted on GD 17 and 19) and survived to the end of the study. Abortions were not observed in any other treatment group. A dose dependent increase in the number of dead or moribund does was observed at dose levels ≥ 200 mg/kg/day. One doe (on GD 16) and eight does (between GD 9-25) died or were sacrificed moribund in the 200 and 400 mg/kg/day treatment groups, respectively. All does in the 600 and 800 mg/kg/day treatment groups were either found dead or sacrificed moribund between GD 8 and 14. Maternal body weight was significantly decreased ($\downarrow 8-31\%$) from controls at dose levels ≥ 200 mg/kg/day.

A non-significant 11% decrease in mean fetal weight was observed in the 200 mg/kg/day treatment group. Although this finding is ostensibly a developmental effect, the declining health of the does at this dose may have contributed to the lower fetal weight. Fetal weight could not be assessed at 400 mg/kg/day because the surviving does exhibited total litter resorptions. The abortions noted above at 200 mg/kg/day and total resorptions at 400 mg/kg/day were considered evidence of both maternal and developmental toxicity given the etiology of these effects are not known. The does from the 400 mg/kg/day treatment group also exhibited significantly decreased gravid uterine weight compared to controls. None of the does from the 600 and 800 mg/kg/day survived to the caesarean. There were no treatment related effects on cesarean parameters nor other evidence of maternal or developmental toxicity in the 50 mg/kg/day treatment group.

The maternal LOAEL is 200 mg/kg/day based on clinical signs of toxicity, abortions, decreased body weight, and mortality. The maternal NOAEL is 50 mg/kg/day.

The developmental LOAEL is 200 mg/kg/day based on abortions and decreased fetal weight. The developmental NOAEL is 50 mg/kg/day.

This non-guideline prenatal developmental study in rabbits is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a prenatal developmental study in rabbits [OCSPP 870.3700b; OECD 414].

COMPLIANCE: A signed and dated Quality Assurance was provided and indicated the study was conducted in compliance with GLP's.

Table 1. Maternal mortality^{ab}						
	Dose in mg/kg bw/day					
	Control	50	200	400	600	800
# dead/total # pregnant does	0/10	0/9 ^c	1/10	8/10	10/10	10/10

^a Data obtained from page 12 in the study report

^b Data presented for pregnant females only.

^c One doe from the 50 mg/kg/day treatment group died on GD 7. Necropsy indicated death was likely due to gavage error and was not treatment related.

Table 2. Mean (±SE) maternal body weight and body weight gain (g)^{ab}						
Gestation Day	Dose in mg/kg bw/day (# of Does)					
	Control (10)	50 (9)	200 (6-10)^d	400 (2-10)	600 (1-10)^e	800 (8-10)^e
Body Weight (g)						
Day 4	3408±97	3351±74	3405±82	3415±86	3445±104	3383±98
Day 6	3464±101	3401±69	3512±77	3452±90	3530±104	3411±111
Day 8	3436±94	3340±64	3233±72	3115±69* (↓9%)	3163±104* (↓8%)	2966±82* (↓14%)
Day 10	3402±93	3330±55	3127±65* (↓8%)	2926±79* (↓14%)	2976±133* (↓13%)	--
Day 12	3413±91	3372±66	3080±60* (↓10%)	2750±67* (↓19%)	2827±207* (↓17%)	--
Day 14	3444±86	3400±80	2999±60* (↓13%)	2599±86* (↓25%)	2765 (↓20%)	--
Day 16	3451±93	3381±73	3039±99* (↓12%)	2500±81* (↓28%)	--	--
Day 18	3429±95	3453±77	3147±128 (↓8%)	2431±62* (↓29%)	--	--
Day 20	3423±104	3458±85	3127±151 (↓9%)	2370±74* (↓31%)	--	--
Day 30	3765±113	3780±68	3738±108	2694±742* (↓28%)	--	--
Body Weight Gain (g)						
Treatment: Days 6-20	-41±72	57±56	-358±124*	-1153±135*	--	--
Gestation: Days 4-30	357±71	428±41	359±68	-751±839	--	--
Gestation (corrected): Day 4-30^c	-195±88	-142±46	-89±60	-761±835	--	--

^a Data obtained from Tables 2a and 2b on pages 21-22 in the study report; values in parentheses are % change from controls calculated by the Reviewer.

^b Data presented for pregnant females only.

^c Gestation maternal body weight gain corrected for gravid uterine weight.

^d Three does from the 200 mg/kg/day treatment group aborted between GD 18-25 and were excluded from the mean body weight calculations.

^e All does in the 600 and 800 mg/kg/day treatment groups died prior to Day 10.

* Statistically different (p <0.05) from the control.

Table 3. Cesarean section observations (mean±SE) ^a						
Observation	Dose (mg/kg bw/day)					
	Control	50	200	400	600 ^b	800 ^b
# Animals assigned (mated)	10	10	10	10	10	10
# Animals pregnant	10	9	10	10	10	10
Pregnancy rate (%)	100	90	80	100	100	80
# Nonpregnant	0	1	0	0	0	0
Maternal wastage						
No. died	0	1	1	8	10	10
No. died pregnant	0	0	1	8	10	10
No. died nonpregnant	0	1	0	0	0	0
No. aborted	0	0	3	0	0	0
No. Premature delivery	0	0	0	0	0	0
No. implantations/doe	9.0±1.1	9.3±0.6	8.9±0.7	8.0±1.0	--	--
No. non-live implantations/doe	0.2±0.1	0.2±0.2	0.7±0.6	8.0±1.0	--	--
Total No. litters	10	9	6	2	--	--
No. live fetuses/doe	8.8±1.1	9.1±0.6	7.5±1.1	-- ^c	--	--
No. dead fetuses/doe	0.0±0.0	0.0±0.0	0.0±0.0	-- ^c		
Resorptions/doe	0.2±0.1	0.2±0.2	0.7±0.6	8.0±1.0	--	--
Litters with total resorptions	0	0	0	2	--	--
Mean fetal weight (g)	49.17±2.80	46.94±0.81	43.75±2.95 (↓11%)	-- ^c	--	--
Gravid uterine weight (g)	552±51	571±28	448±66	10±4*	--	--


^a Data obtained from Table 4, page 26 in the study report; values in parentheses are % change from controls calculated by the Reviewer.

^b No caesarean section data due to total group loss

^c The two surviving does from the 400 mg/kg/day group exhibited total litter resorptions.

* Statistically different (p <0.05) from the control.

EPA Reviewer: Austin Wray
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Chronic oral toxicity study; dietary- rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (96% a.i.)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Hard GC and GE Neal. 1992. Sequential study of the chronic nephrotoxicity induced by dietary administration of ethoxyquin in Fischer 344 rats. Fundamental and Applied Toxicology. 18: 278-287. MRID 50920704.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a chronic oral toxicity study (MRID 50920704), ethoxyquin (96% a.i.; Sigma Chemicals) was administered in the diet at concentrations of 0 or 0.5% ethoxyquin, (equivalent to 65.3/70.6 mg/kg/body weight for males/females as reported in the publication) to 6-20 Fischer-344 rats/sex/dose/sacrifice for 4 weeks to 18 months. Another group received the ethoxyquin diet for 24 weeks then a control diet for 34 weeks to assess recovery. Feed consumption and body weight were monitored. Animals were killed at selected times (4 weeks, 12-14 weeks, 24 weeks, 58 weeks and 18 months) and kidneys were examined histopathologically.

Treated rats had lower body weight gains than control rats (80%/ 90% of controls for males/females), but feed consumption did not differ significantly between the groups. Interstitial degeneration of the extremity of the renal papilla was seen in male rats after 4 weeks. After 12 to 14 weeks, the papillary lesion was more advanced and papillary necrosis and pyelonephritis were seen; two of eight treated females showed a slight focal loss of interstitial detail at the papillary tip. After 24 weeks, nearly all male rats (9/10) exhibited complete papillary necrosis with loss of part of the papilla and foci of mineralization in the abscission layer. Pyelonephritis and urothelial hyperplasia were also seen in male rats after 24 weeks of exposure. All treated male rats (9/9) at 58 weeks had papillary necrosis, pyelonephritis, urothelial hyperplasia, and moderately severe chronic progressive nephropathy. At 18 months, 18 of the 19 treated males had complete papillary necrosis with tissue loss and exhibited urothelial hyperplasia, and one of 19 males presented with interstitial degeneration of the papillae without tissue loss. All treated males exhibited pyelonephritis and severe end stage chronic progressive nephropathy. Although the incidence was greater at this time point, the authors indicate there was evidence of healing in the truncated papillae, there were fewer foci of active inflammation, fewer basophilic

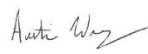
hyperplastic tubules associated with pyelonephritis, and less marked hyperplasia with papillary necrosis compared to previous sampling times.

Kidney damage was less extensive in the females. Papillary degeneration in female rats was not observed until 12 to 14 weeks of exposure and the incidence increased with exposure duration. At 58 weeks, eight of nine females had varying degrees of papillary tip interstitial degeneration, five exhibited mild pyelonephritis, and one exhibited low-grade urothelial hyperplasia. By 18 months, all treated females (13/13) had interstitial degeneration of the papillary tip, but the lesion had not progressed beyond the earlier stage. Unlike males, none of the treated females exhibited papillary necrosis throughout the exposure period. Mild urothelial hyperplasia was also observed in two of 13 females at 18 months, but there were no signs of pyelonephritis in females at this point. The authors concluded that ethoxyquin is a potent inducer of renal papillary necrosis in male rats and that female rats are far less susceptible to its effects. Furthermore, they considered the pyelonephritis and urothelial hyperplasia to be secondary response related to the papillary necrosis and not a direct reaction to ethoxyquin.

This non-guideline chronic study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSPP 870.4100; OECD 452].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Prenatal developmental toxicity study - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Santoquin (67% a.i.)

SYNONYMS: Ethoxyquin; 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Khera KS, C Whalen, G Trivett, and G Angers. 1979. Teratologic assessment of maleic hydrazide and diaminozide, and formulations of ethoxyquin, thiabendazole and naled in rats. Journal of Environmental Science & Health Part B. 14(6): 563-577. MRID 50920705.

SPONSOR: None reported.

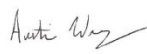
EXECUTIVE SUMMARY: In a prenatal developmental toxicity study (MRID 50920705), an ethoxyquin formulation, Santoquin (67% a.i. and 33% unknown ingredients; Lot# 417; Monsanto), was administered to 20 female Wistar rats/dose via oral gavage (in corn oil vehicle) at dose levels of 0, 125, 250, or 500 mg formulation/kg/day (0, 83.8, 168, or 335 mg a.i./kg/day after adjusting for purity) from gestation day (GD) 6 through 15.

There were no clinical signs of toxicity in dams in any of the treatment groups nor treatment related effects on maternal and pup body weight, number of pregnant females, number of corpora lutea, total implantations, resorptions, dead fetuses, live fetuses, male/female ratio. A significant increase in the number of fetuses with anomalies was noted in the 250 mg formulation/kg/day treatment group compared to controls. Although there was a concordant increase in the number of litters containing fetuses with anomalies in this treatment group compared to controls, it was not statistically significant nor was there a dose response for anomalies based either on individual fetuses or on the litter when examining the other treatment groups. Consequently, this finding was not considered treatment related. The study authors did not present the litter incidence for individual anomalies; however, there were no significant differences in the fetal incidence for individual anomalies compared to controls.

This non-guideline prenatal developmental toxicity study is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSPP 870.3700a; OECD 414].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity study; dietary- rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (90% a.i.)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Manson MM, JA Green, BJ Wright, and P Carthew. 1992. Degree of ethoxyquin-induced nephrotoxicity in rat is dependent on age and sex. Arch Toxicol. 66: 51-56. MRID 50920706.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 50920706), groups of 4-8 male rats received a diet containing ethoxyquin (90% a.i.; Sigma Chemicals) at 0 or 0.5% (5000 ppm; equivalent to 250 mg/kg/day assuming 1 ppm = 0.05 mg/kg/day in rats) from 3 or 8 weeks of age for 20, 26, or 30 weeks. In addition, eight female rats received a diet containing either 0 or 5000 ppm (250 mg/kg/day) of the same ethoxyquin test article for 26 weeks from 8 weeks of age.

Female rats were much less susceptible to the toxic effects of ethoxyquin than males of the same age. Treated males and females both exhibited significantly decreased body weight (\downarrow 10-15%) compared to controls at the end of 20, 26, and 30-week exposures. Males exhibited an increase in absolute kidney weight (\uparrow 15-54%) and both sexes exhibited a significant increase in relative kidney weight (\uparrow 15-79%), with a greater increase observed in males.

In treated males, damage to the cortex (eosinophilic cytoplasmic inclusions in tubular epithelial cells, protein accumulation in lumina of tubules, thickening of basement membranes around tubules and Bowman's capsules and hyperplasia of pelvic transitional epithelium) was similar in both age groups and was observed after 20, 26, and 30 weeks of exposure. Treated males also exhibited an increase in BrdU labelling in epithelial tubule cells both in regenerating basophilic tubules typical of aging lesions and in mildly hyperplastic tubules. In addition to the cortex damage, male rats exposed to ethoxyquin as weanlings (e.g. 3 weeks old at start of exposure) suffered from extensive papillary necrosis and one animal exhibited severe calcification in the papilla. Pyelonephritis was also observed in two males from the weanling treatment groups but was considered incidental by the study authors and attributed to bacterial infection. There was little evidence of papillary necrosis in older males (8-weeks of age at the start of exposure). Two (out of four) older males exposed for 20 weeks exhibited a small amount of necrosis in the

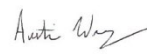
interstitial cells of the papilla tip, and one older male exhibited papillary necrosis and another loss of interstitial cells at papillary tip after 30 weeks of exposure. No evidence of papillary necrosis or calcification was observed in older males exposed for 26 weeks. Older males exposed for 20 weeks also exhibited a reduction or complete loss of GGT enzyme activity in a number of tubules. Eight-week-old female rats exposed for 26 weeks did not exhibit evidence of papillary necrosis or damage to the cortex but did have brown pigment in tubule cells that was also observed in males and was previously identified as lipofuscin. Male rats were also more prone than females to proteinuria, which was greatly exacerbated by ethoxyquin in both age groups.

Overall, there is very little evidence of nephrotoxicity in older female rats after exposure to ethoxyquin at 0.5% in the diet for 26 weeks. In males, the initial age of the animal, as well as the length of treatment, influenced the extent of toxicity observed in the kidneys.

This non-guideline subchronic study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSPP 870.3100; OECD 408].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity study; dietary- rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (>95% a.i.)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Manson MM, JA Gree, and HE Driver. 1987. Ethoxyquin alone induces preneoplastic changes in rat kidney whilst preventing induction of such lesions in liver by aflatoxin B₁. Carcinogenesis. 8(5): 723-728. MRID 50920707.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a subchronic rat oral toxicity study (MRID 50920707), pretreatment of 7-8-week-old male Fischer 344 rats with the antioxidant ethoxyquin (>95% pure; Koch Light Laboratories), followed by administration of aflatoxin B₁ (AFB₁) in the continuing presence of ethoxyquin was used to examine the effect of the antioxidant on liver and kidney. Groups of 8-10 mice were exposed to one of the following regimens: 1) control diet; 2) single i.p. injection of 0.25 mg/kg AFB₁ followed by diet containing 1 ppm AFB₁; 3) pretreatment with diet containing 0.5% ethoxyquin (5000 ppm equivalent to 250 mg/kg/day assuming 1 ppm = 0.05 mg/kg/day in rats), single i.p. injection of 0.25 mg/kg AFB₁ followed by diet containing 1 ppm AFB₁ and 0.5% ethoxyquin; and 4) control diet containing 0.5% ethoxyquin. Animals were maintained on their respective diets for 23 weeks until sacrifice. After euthanasia, the liver and kidneys were removed, weighed, and prepped for histology. Body weight was also measured at study termination.

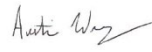
Body weight was significantly decreased from controls in the AFB₁/ethoxyquin treatment group; however, there was no treatment-related adverse change in body weight in the ethoxyquin only treatment group. Liver weight was significantly elevated above controls (↑16-44%) in both ethoxyquin treatment groups at the end of the 23-week period. Ethoxyquin in the diet completely prevented the formation of AFB₁-induced preneoplastic liver lesions as judged by morphological alteration, or by markers such as gamma glutamyl transpeptidase (GGT), glutathione S-transferase P (GST-P) or J1, an unknown membrane-bound antigen. Although there was an observed significant increase in hepatic GGT and evidence of a slight zonal elevation of GST-P activity in periportal areas, no preneoplastic lesions were observed in the liver of rats on the ethoxyquin only diet. While protection was afforded to the liver, ethoxyquin alone after 23 weeks of feeding at 0.5% in the diet caused severe damage to the kidney. Kidney weight was not significantly different from controls, but ethoxyquin treated animals did exhibit characteristics of

chronic glomerulonephritis, that are normally observed in older animals. In addition, hyperplastic and putative preneoplastic tubules were visible with many containing brown pigment (staining suggested it was lipofuscin) and mitotic figures. Pigment-bearing hyperplastic tubules exhibited GB-ase activity and a reduction or complete loss of GGT activity. GST-P, usually localized to specific areas of cortex, was extended to the whole cortex in ethoxyquin treated animals and exhibited variable activity in basophilic tubules and tubules with flattened epithelia. All kidney findings were presented qualitatively without details on the incidence or severity and numerical values were not presented for the enzyme activity assessments in the kidneys.

This non-guideline subchronic study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSPP 870.3100; OECD 408].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph. D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity study; dietary- rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (90% a.i.)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Neal GE, DJ Judah, GG Hard, and N Ito. 2003. Differences in ethoxyquin nephrotoxicity between male and female F344 rats. Food and Chemical Toxicology. 41: 193-200. MRID 50920708.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a subchronic rat oral toxicity study (MRID 50920708), groups of Fisher 344 rats were administered ethoxyquin (90% a.i. and 10% oxidized form of ethoxyquin; Sigma Chemical) in the diet at 0, 0.01, 0.05, 0.01, 0.25 or 0.5% (equivalent to 0, 5, 25, 50, 125, or 250 mg/kg/day assuming 1 ppm = 0.05 mg/kg/day in rats) for 3 or 6 months (5 males/group for 3 months feeding; 8/sex/group in the 6-month feeding except in the 0.05, 0.1, and 0.25% female groups where 5 females/group were used). At the end of the feeding period, animals were sacrificed, and kidneys removed for histological examination.

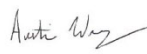
Dietary intake did not vary significantly between the groups. There was no indication in the article that body weights were recorded. No mortalities were reported in any group. Interstitial degeneration of the papilla or frank papillary necrosis was evident in male rats after 3 (1/5 and 3/5 males, respectively) and 6 months (4/8 and 4/8 males, respectively) of exposure to 0.5% in the diet, but not in male rats provided a diet with 0.25% ethoxyquin or lower dietary concentrations. In females, early minimal interstitial degeneration was observed in one rat only at the 0.5% exposure level after 6 months of exposure. A slight increase in lysosome distribution in proximal tubules was observed in male rats receiving 0.25 and 0.5% of ethoxyquin particularly at 3 months but also at 6 months. There was no evidence of hyaline droplet accumulation in these animals. Urothelial hyperplasia was observed in the four males exhibiting papillary necrosis in the 0.5% diet group after 6 months of exposure. Urinary protein (albumin) was elevated equally in both males and females at the 0.5% dietary level of ethoxyquin, however papillary necrosis was male specific. When ¹⁴C-ethoxyquin was administered intraperitoneal or orally by gavage (10 mg/kg), the radiolabel was associated with urinary albumin and not alpha 2 globulin (α_{2μ}-g). The ratio of the excreted radioactivity in the urine to feces was 7.3-8.2. Autoradiographic sections of the kidneys did not show differences among the sexes in the distribution of the radioactivity or retention of ethoxyquin. Fecal and urinary metabolites'

profiles were similar in the two sexes. The study authors concluded that the sharp threshold of toxicity in the male rat could indicate a fine balance between toxifying/detoxifying metabolism of ethoxyquin. The presence of a polar metabolite(s), immobile on TLC, was detected in both fecal extracts and urine samples from males and females. No unmetabolized ethoxyquin was detected in any sample. Treatment of the urine and fecal extracts with β -glucuronidase failed to produce any detectable level of non-polar labeled compounds.

This non-guideline subchronic study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSPP 870.3100 OECD 408].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph.D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (90% a.i.)
3-[¹⁴C]-ethoxyquin (>96% radiolabeled purity)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Sanders JM, Burka LT, and Matthews HB. 1996. Comparative metabolism and disposition of ethoxyquin in rat and mouse. I. Disposition. Xenobiotica. 26(6): 583-595. MRID 50920709.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a metabolism study (MRID 50920709), [¹⁴C]-ethoxyquin (>96% radiolabeled purity; Amersham Corporation) diluted with unlabeled ethoxyquin (90% a.i.; Sigma Chemical Co.) in a vehicle (1:1:8 ratio of ethanol/emulphor EL-620/water) was administered to male F344 rats and B6C3F1 mice (three rodents/treatment) as a single dose via gavage at 2.5 (rats only), 25, or 250 mg/kg or intravenous (i.v.) injection at 25 mg/kg. Urine, feces, and expired air was collected and analyzed at times points from 4 to 72 hours post dosing. A subset of animals from the gavage (24 and 72 hours) and i.v. (0.25 to 24 hours) treatment groups were euthanized at various timepoints after exposure for tissues distribution analysis. Radioactivity was quantified in the bile and blood/plasma in separate experiments. For bile analysis, three F344 bile cannulated rats were exposed to a single i.v. dose of 25 mg/kg and bile was collected for 6 hours after administration. For blood/plasma analysis, four vein cannulated rats/treatment were exposed to a single dose of 25 mg/kg either via gavage or i.v. and blood was collected from 0.08 to 24 hours after administration. A final experiment was conducted to evaluate bioaccumulation. [¹⁴C]-ethoxyquin was administered to F344 rats (four rats/treatment) via gavage either as a single dose or six daily doses of 25 or 250 mg/kg. Urine and feces were collected every 24 hours and animals were euthanized 24 hours after the last dose for tissue distribution analysis. All samples were analyzed for ethoxyquin and metabolites via HPLC

Ethoxyquin was rapidly absorbed into circulation and efficiently eliminated in both rats and mice following oral exposure to 25 mg/kg. The study authors report (but did not show data to confirm) that blood concentration peaked within 1 hour of exposure in rats. Approximately 60% of the total radioactivity in the blood partitioned to plasma. Based on the i.v. experiment, the distribution from circulation to tissues was rapid with peak tissue concentrations observed within 15 minutes and initial distribution was primarily to adipose, liver, and kidney tissue. At 24 hours

post dose, ethoxyquin concentration was highest in the liver (1-1.3 and 1.1-1.5% of the administered dose in 25 mg/kg oral and i.v. exposed animals, respectively) and adipose tissue (0.6-1.7 and 0.9-6.4% of the administered dose in 25 mg/kg oral and i.v. exposed animals, respectively). Tissue distribution in mice and rats was mostly similar except for adipose tissue which peaked later and higher in mice and was eliminated more rapidly post-dose.

Elimination was rapid in both species after exposure to 25 mg/kg irrespective of route of administration. Based on i.v. experiment, elimination half-life was 23 minutes in plasma. By 24 hours post dose 79-100% of the administered dose was eliminated, with a majority excreted in the urine. Radioactivity in expired air was negligible. A general dose proportionality in tissue distribution and elimination was observed across the 2.5-25 mg/kg range in rats. Dose proportionality in tissue distribution (except for adipose tissue) was evident in the 25-250 mg/kg range in both species; however, cumulative elimination at 24 hours post-dose was decreased by greater than 30% at the 250 mg/kg compared to 25 mg/kg. Most of the unexcreted radioactivity in high dose animals was found in the gastrointestinal tract. An additional 20% of the administered dose was excreted during the second day in high dose animals but only trace amounts were excreted thereafter. Generally, urine/fecal elimination patterns were similar for oral and i.v. exposure in both species suggesting that biliary elimination was contributing to the fecal radioactivity. The bile-cannulated rat experiment confirmed that greater than 40% of the i.v. administered 25 mg/kg dose was excreted in the bile within 6 hours of exposure.

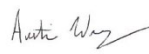
Repeat oral dosing of 25 mg/kg in rats lead to a 2-3-fold increase in radioactivity found in major tissues (liver, kidney, muscle, skin, and adipose tissue) and blood compared to animals exposed to a single dose. A similar finding was also noted in animals exposed to 250 mg/kg; however, the increase in concentration from repeat dosing was significant in blood, skin, and adipose tissue only and were not dose proportional. Less radioactivity was observed in the stomach of animals exposed to multiple doses of 250 mg/kg compared to single dose. Elimination of the 25 mg/kg dose was consistent regardless of the number of doses. In contrast, urine excretion increased for 3-4 days in the 250 mg/kg group, ultimately reaching a steady state proportional to the daily excretion observed in the 25 mg/kg treatment group.

Ethoxyquin was rapidly and almost completely metabolized in both species. Little to no parent compound was observed in rat liver, kidney and adipose tissue collected 15 minutes after i.v. exposure to 25 mg/kg. Only trace amounts of parent compound were observed in the feces and none was detected in the urine.

This non-guideline metabolism study in rats and mice is classified **Acceptable** and is not intended to fulfil the guideline requirement for a metabolism study [OCSPP 870.7485, OECD 417].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): 5,7-¹⁴C-ethoxyquin (98% radiochemical and chemical purity)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Skaare JU and I Nafstad. 1979. The distribution of ¹⁴C-ethoxyquin in rat. Act Pharmacol et Toxicol. 44: 303-307. MRID 50920710.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a metabolism study (MRID 50920710), 5,7-¹⁴C-ethoxyquin (98% radiochemical and chemical purity; Radiochemical Centre) was diluted in arachis oil and administered to male albino rats via oral intubation at a dose of 104 mg/kg (250 µCi/kg). Rats (2/time point) were sacrificed at 0.5, 1, 2, 4, 8, 10, 12, 16, 20, 24, 48, and 144 hours after exposure, frozen, and sectioned for whole body autoradiography. Liver samples were also collected and analyzed for radioactivity. The results of the whole body autoradiography study were presented qualitatively (i.e. as images) whereas quantitative data were presented for the liver samples.

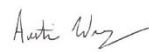
Whole body autoradiography of exposed rats demonstrated that ethoxyquin radioactivity distributed to the blood and nearly all tissues within 0.5 hours. The highest activity was noted in the gastrointestinal tract, liver, and kidney (renal pelvic cavity and renal cortex) with minor amounts in the lung and skin. Radioactivity in tissues continued to increase, particularly in adipose tissue, with time after exposure and peaked at 4 to 8 hours. Tissue radioactivity levels remained consistent from 4 to 20 hours after exposure and visibly declined in most tissues by 48 hours with the exception of the gastrointestinal tract, medulla of the kidney, and blood. At 144 hours, high radioactivity was still evident in the renal cortex and moderate to weak radioactivity was observed in the liver, intestines, lung, and heart blood. No radioactivity was observed in the central nervous system at any time point.

Radioactivity levels quantified in the liver followed the qualitative temporal tissue distribution pattern noted in the whole-body autoradiography data. Radioactivity in the liver was observed as early as 0.5 hours after exposure (2.2% of the administered dose) and peaked (2.9% of the administered dose) between 4-8 hours post dose. The radioactivity levels reduced to 41% of peak value (1.0% of the administered dose) at 24 hours and down to 7.5% of the peak value (0.2% of the administered dose) by 144 hours.

This non-guideline metabolism study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a metabolism study [OCSPP 870.7485, OECD 417].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (a.i. % not reported)
[¹⁴C]-ethoxyquin (98% radiochemical and chemical purity)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Skaare JU and E Solheim. 1979. Studies on the metabolism of the antioxidant ethoxyquin, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline in the rat. Xenobiotica. 9(11): 649-657. MRID 50920711.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a metabolism study (MRID 50920711), ethoxyquin (Koch-Light, authors describe it as pure but do not provide % a.i.) and [¹⁴C]-ethoxyquin (98% radiochemical and chemical purity) was administered as a single dose via gavage in a suspension of soya oil to male albino rats (3 rats/dose) at doses of 100 and 400 mg/kg or 100 mg/kg (200 µCi/kg), respectively. Expired air, urine and feces were separately collected for 24-hour periods up to 6 days after exposure and analyzed for radioactivity. In addition, the urine samples collected at 24 hours were analyzed for ethoxyquin metabolites.

Ingested ethoxyquin was rapidly eliminated. Approximately 67-80% of the administered 100 mg/kg dose was collected in excreta at 24 hours post-dose, with the majority (50-57% of the administered dose) excreted in the urine. Fecal excretion accounted for 17-23% of the administered dose at 24 hours. Fecal elimination tended to be higher than urine elimination at 48 and 72 hours post dose; however, overall it was a minor elimination pathway. The amount of ethoxyquin released in expired air was negligible (<1% of the administered dose). By 6 days post dose approximately 95% of the administered dose was eliminated.

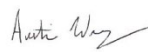
Seven metabolites and unchanged parent were detected in the urine. The major metabolites were de-ethylated ethoxyquin (6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline) and its chemical oxidation product (2,2,4-trimethyl-6-quinolone). The study authors report that absolute quantification of the major metabolites was hindered due to an inability to purify the reference standard. However, the study authors estimate 6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline accounted for 40-50% of the dose excreted in urine in the 24-hour period after exposure. Minor metabolites included dihydroxylated ethoxyquin and four isomers of hydroxylated ethoxyquin (isomers were not individually identified by the study authors). Parent was also a minor

contributor to the radioactivity quantified in the urine. The study authors indicate that no attempt was made to quantify the minor metabolites nor parent because it was estimated that they were excreted in small amounts compared to the major metabolites.

This non-guideline metabolism study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a metabolism study [OCSPP 870.7485, OECD 417].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph. D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin (a.i. % not reported)
[¹⁴C]-ethoxyquin (98% radiochemical and chemical purity)

SYNONYMS: 6-ethoxy-1,2,-dihydro-2,2,4-trimethylquinoline

CITATION: Skaare JU. 1979. Studies on the biliary excretion and metabolites of the antioxidant ethoxyquin, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline in the rat. Xenobiotica. 9(11): 659-668. MRID 50920712.

SPONSOR: None reported.

EXECUTIVE SUMMARY: In a metabolism study (MRID 50920712), ethoxyquin (Koch-Light, authors describe it as pure but do not provide % a.i.) and [¹⁴C]-ethoxyquin (98% radiochemical and chemical purity) was administered as a single oral dose in arachis oil to bile duct cannulated male albino rats (6 rats/treatment group) at a dose of 100 mg/kg (25 µCi/kg). Two treatment groups received a continuous intragastric infusion of either 0.9% NaCl (Group 1) or sodium taurocholate dissolved in saline (Group 2). Ethoxyquin was administered to rats from Group 1 and 2 at 24 hours after bile-duct cannulation and bile was collected hourly up to 12 hours after exposure and at 24 and 48 hours post exposure. Lymph was also collected hourly up to 12 hours after intragastric exposure and at 24 and 48 hours post exposure from 2 rats to quantify intestinal lymphatic absorption. A third group (Group 3) did not receive any fluid infusion, were exposed to the compound 2 hours after bile duct cannulation, and bile was collected at 2 to 48 hours after exposure. Bile samples from all groups were analyzed for radioactivity and metabolite identification. The reviewer notes that the methods section indicate the fluid infusion and ethoxyquin exposure in the Group 1 and 2 rats was via ventricular catheter whereas other sections described the exposure as intragastric. Although there is an apparent discrepancy, it is assumed that the exposure in this study is via the oral route.

Rats from Group 1 and 2 exhibited similar biliary excretion patterns following ethoxyquin exposure despite receiving different fluid infusions that had a slight impact on bile flow. Biliary excretion in these groups accounted for 26-33% and 33-42% of the administered radioactivity at 12 and 24 hours post exposure, respectively. A range for biliary excretion at 48 hours for Groups 1 and 2 was not reported in the text but appears to average ~35-40% of the administered radioactivity based on Figure 1 in the publication. In contrast, bile flow and cumulative biliary excretion of ethoxyquin was more variable in the Group 3 rats with between 9-30% of the

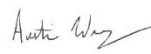
administered radioactivity excreted in the bile at 12 hours post exposure. A range for biliary excretion at 24-48 hours for Group 3 was not reported in the text but appears to average ~40 and ~50% of the administered radioactivity at 24 and 48 hours, respectively, based on Figure 1 in the publication. Absorption across the intestine by the lymphatic route was low (~3% within 24 hours).

Six metabolites and unchanged parent were detected in the bile. The metabolic pathway proposed by the study authors is presented below. The major component of the bile was unchanged parent which accounted for 75-85% of the radioactivity excreted in the bile 12 hours after dosing. The six metabolites were all minor components compared to unchanged parent. The minor biological metabolites identified were hydroxylated ethoxyquin and dihydroxylated ethoxyquin. Although another biological metabolite, de-ethylated ethoxyquin (6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline), was not identified in the bile, the presence of its chemical oxidation product 2,2,4-trimethyl-6-quinolone suggest that it may have been present. The remaining identified minor metabolites were chemical oxidation products of ethoxyquin and its biological metabolites. The chemical oxidation products include 6-ethoxy-2,4-dimethyl-quinoline, 6-ethoxy-2,2,4-trimethyl-8-quinolone, hydroxylated 6-ethoxy-2,2,4-trimethyl-8-quinolone, and 2,2,4-trimethyl-6-quinolone.

This non-guideline metabolism study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a metabolism study [OCSPP 870.7485, OECD 417].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray, Ph. D.
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity study; dietary- rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin dimer (98% dimer)

SYNONYMS: 8-(6-ethoxy-2,2,4 trimethyl-1,2-dihydro-1-quinolinyl)-6-ethoxy-2,2,4 trimethyl-1,2-dihydroquinoline)

CITATION: Ørnsrud R, A Arukwe, V Bohne, N Pavlikova, and A-K Lundebye. 2011. Investigations on the metabolism and potentially adverse effects of ethoxyquin dimer, a major metabolite of the synthetic antioxidant ethoxyquin in salmon muscle. Journal of Food Protection. 74(9): 1574-1580. MRID 50938001.

SPONSOR: The Norwegian Research Council and The Fishery and Aquaculture Industry Research Fund


EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 50938001), ethoxyquin dimer (EQDM; 98% EQDM; Synthetica AS) was administered to eight male F344 rats/dose in the diet at dose levels of 0 or 12.5 mg EQDM/kg/day for 90 days. Tissue samples (liver, kidney, and adipose tissue) were taken to evaluate distribution of EQDM following oral exposure. In addition, body and organ weight, clinical chemistry markers for kidney and liver function, gene expression, and enzyme activity were assessed.

There were no clinical signs of toxicity in the treatment group nor treatment related changes in body weight, liver and kidney function, or organ weight. Of the three tissues sampled, EQDM tissue burden was highest in adipose tissue followed by the kidneys and liver. Gene expression and enzyme activity in the liver was affected by EQDM exposure. Liver *Cyp1a1* mRNA was significantly reduced, and liver *Cyp2b1* and *Gstpi1* mRNA were significantly increased in the treated rats whereas *Cyp3a62* and *Ugt1a* mRNA levels were comparable with controls. Both glutathione S-transferase levels and activity were lower in the treatment group compared to controls. In contrast, liver cytochrome P-450 (measured in the EROD, BROD, MROD, BFCOD, and PROD assays) and uridine diphosphate glucuronyl transferase activity in treated rats were similar to controls.

This non-guideline subchronic study in rats is classified **Acceptable**. The study was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSPP 870.3100; OECD 408].

COMPLIANCE: Not reported.

EPA Reviewer: Austin Wray
RABIV, Health Effects Division (7509P)

Signature: 
Date: 09/17/19

TXR#: 0057944

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity study; dietary- rat; Non-guideline.

PC CODE: 055501

DP BARCODE: D454047

TEST MATERIAL (PURITY): Ethoxyquin dimer (EQDM; 98% dimer)
Ethoxyquin (99% a.i.)

SYNONYMS: EQDM: 8-(6-ethoxy-2,2,4 trimethyl-1,2-dihydro-1-quinoliny)-6-ethoxy-2,2,4 trimethyl-1,2-dihydroquinoline)

CITATION: Bernhard A, JD Rasinger, H Wisløff, Øyvør Kolbjørnsen, LS Myrmel, MHG Berntssen, A-K Lundebye, R Ørnsrud, and L Madsen. 2018. Subchronic dietary exposure to ethoxyquin dimer induces microvesicular steatosis in male BALB/c mice. Food and Chemical Toxicology. 118: 608-625. MRID 50938002.

SPONSOR: Norwegian Seafood Research Fund, the Marine Ingredients Organisation, Marine Harvest ASA, EWOS AS/Cargill Aqua Nutrition, Biomar AS, Skretting AS, and Europharma

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 50938002), ethoxyquin dimer (EQDM; >95% EQDM; Synthetica AS) was administered to 80 male BALB/c mice/dose in the diet at dose levels of 0, 0.1, 1, 100, 1000, 3000, or 5000 ppm (equivalent to 0, 0.015, 0.081, 10, 99, 286, or 518 mg EDQM/kg/day) for 90 days. Body weight was measured weekly and whole body composition was assessed at the initiation of the study and at 12 weeks. Blood and tissue samples (heart, spleen, liver, kidney, brain, and adipose tissue) were collected after sacrifice and collected tissues were weighed. Spleen, liver and kidney tissues were also analyzed for EQ and EDQM content and examined under a microscope. Metabolomics and proteomics analyses were conducted on the liver as well as an assessment of the redox status and lipid classes.

There were no clinical signs of toxicity in the treatment group nor treatment related changes in body weight. At doses ≥ 99 mg EDQM/kg/day, mice exhibited symptoms of liver toxicity including significant increases in plasma ALT levels, hepatic GSSG, liver weight, and total liver lipid, as well as an increase in the incidence of steatosis and increase in incidence and severity of single cell necrosis in the liver. Exposure to doses ≥ 99 mg EDQM/kg/day also significantly altered the hepatic metabolite profile, with the most prominent changes related to lipid metabolism. The hepatic protein profile exhibited a concordant change at similar dose levels most notably in protein involved in pathways associated with mitochondrial function, amino acid

degradation, energy metabolism, xenobiotic metabolism, estrogen biosynthesis, and fatty acid beta-oxidation. At doses ≥ 286 mg EQDM/kg/day, additional effects were observed including a significant decrease in adipose tissue weight, significant increase in liver triacylglycerides, and a significant increase in hepatic GSH. Similar evidence of liver toxicity was not observed at doses ≤ 10 mg EQDM/kg/day nor was there any indication of increased organ weight or histopathological lesions in the other tissues examined.

In a parallel treatment group, ethoxyquin (EQ; 99% purity; Industrial Técnica Pecuaria) was initially supplied in the diet at 5000 ppm (estimated at 750 mg/kg/day assuming 1 ppm is equivalent to 0.15 mg/kg/day in mice). The study authors noted a potential palatability issue with the initial ethoxyquin dietary concentration level that resulted in mice refusing feed and substantial body weight loss during the first ten days. The dietary concentration was reduced to 1000 ppm (estimated at 150 mg/kg/day assuming 1 ppm is equivalent to 0.15 mg/kg/day in mice) for the rest of the 90-day exposure period which resolved the palatability issue; however, the mice did not fully recover. Although this parallel group was intended as a point of comparison for the toxicity observed in the EDQM treated mice, the body weight decrements due to palatability confounded interpretation of the effects observed in this treatment group. As a result, the study authors did not present the data for the ethoxyquin only experimental group in the publication.

This non-guideline subchronic study in rats is classified **Acceptable**. The study was conducted in accordance with OECD guideline 408; however, it was not intended to fulfil the guideline requirement for a subchronic study in rats [OCSPP 870.3100; OECD 408].

COMPLIANCE: Not reported.